REMARKS

Favorable reconsideration of the subject application is respectfully requested in view of the above amendments and the following remarks. Claims 1-112 are pending in the instant application, and claims 42-44 and 47-57 are currently under consideration. Claims 42, 43, 47 and 51 have been amended and claim 113 has been added to more particularly point out and distinctly claim certain subject matter of the invention; claims 1-41, 44-46 and 58-112 have been canceled. Support for these amendments may be found throughout the specification and claims as originally filed, and it is urged that the amendments do not constitute new matter. Support for human ANT1 variants having at least 95% identity to SEQ ID NO:31 is provided, *e.g.,* at page 24, lines 4-6, at page 23, lines 3-25, and at page 21, lines 1-19, and support for human ANT1 fragments comprising at least 30 amino acid residues of SEQ ID NO:31 is provided, *e.g.,* on page 24, lines 6-8. It should also be noted that the above amendments are not to be construed as acquiescence with regard to any of the Examiner's rejections, and are made without prejudice to prosecution in a related divisional, continuation or continuation-in-part application of any subject matter removed or modified by the present amendment.

RESPONSE TO RESTRICTION REQUIREMENT

In response to the restriction requirement, Applicants hereby elect Group IV, claims 42-44 and 47-57, drawn to ANT1 polypeptides, classified in class 530, subclass 300, for examination at this time.

OBJECTION TO THE DISCLOSURE

The Action objects to the disclosure for allegedly containing informalities in not providing sequence identifiers for the sequences listed in Figures 1A, 1B, and 2.

Applicants have amended the Brief Description of the Drawings to reference the appropriate sequence identifiers, as indicated above. Applicants respectfully request that this basis of objection be withdrawn in light of this amendment.

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 43-45 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Specifically, the PTO asserts that claim 43 is unclear in light of dependent claims 44-45 directed to variants and fragments of the polypeptides recited in claim 43. In addition, the PTO alleges that the scope of claim 43 is ambiguous and that claims 43 and 44 are indefinite where it is unclear whether they are proper dependent claims.

Applicants respectfully traverse this rejection. Applicants are puzzled that the Action at page 10, last line, asserts that "Claim 43 appears to encompass full-length ANT2" when claim 43, as amended according to the Preliminary Amendment submitted with the present application at the time of filing, recited "an isolated human ANT1 adenine nucleotide translocator polypeptide". Given that the specification clearly describes the three human ANT isoforms ANT1, ANT2 and ANT3 (*e.g.*, at page 18, lines 5-22), it is difficult to find support for the PTO's assertion that claim 43, directed to a human ANT1 polypeptide, would appear to encompass "full-length ANT2". Also confusing is the rejection of claim 45, where Applicants note that claim 45 was withdrawn from further consideration by the Examiner, as being drawn to a non-elected invention.

Nevertheless, solely for purposes of advancing prosecution of the application and without acquiescence in any rejection, Applicants have canceled claims 44 and 45 without prejudice, thereby obviating the rejection of these claims. In addition, claim 43 has been amended to more particularly point out and distinctly claim what Applicants regard as the invention, and to make explicit what was implicit. Hence, the claimed isolated human ANT1 polypeptide is a *recombinant* human ANT1 polypeptide having an amino acid sequence set forth in SEQ ID NO:31, or a variant thereof having at least 95% sequence identity to the sequence set forth in SEQ ID NO:31. (*see, e.g.,* specification at page 24, lines 4-8, at page 23, lines 3-25, and at page 21, lines 1-19.)

Accordingly, Applicants respectfully submit that the application is in full compliance with the requirements of 35 U.S.C. § 112, second paragraph, and request that these rejections be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 102

Claims 43-45 stand rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Marzo *et al.* (1998 *Science* 281:2027). Specifically, the PTO asserts that Marzo *et al.* disclose purified human ANT2 proteins, which are considered variants and fragments of a recombinant ANT1 polypeptide.

Claims 43-45 also stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Adrian *et al.* (1986 *Mol. Cell. Biol.* 6:626). The PTO asserts that Adrian *et al.* disclose the expression of yeast ANT-β-gal fusion proteins, which are considered variants of recombinant human ANT1.

Claims 43 and 44 stand further rejected under 35 U.S.C. § 102 (e) as allegedly being anticipated by Wallace *et al.* (U.S. No. 6,013,858). More specifically, the PTO asserts that Wallace *et al.* disclose the synthesis of an ANT1 peptide that is patentably indistinguishable from the fragment of recombinant ANT1 encompassed by claims 43 and 44.

As an initial matter and as also discussed above, Applicants note that claim 45 was previously withdrawn by the Examiner as being directed to non-elected subject matter, and that claims 44 and 45 are canceled by the amendment submitted herewith, thereby obviating rejections of these two claims.

Applicants respectfully traverse the rejection of claim 43 and submit that because the publications cited by the PTO fail to disclose each and every element of the instant claim, no *prima facie* case of anticipation has been established. The present invention is directed to an isolated human ANT1 polypeptide that is a recombinant human ANT1 polypeptide having an amino acid sequence set forth in SEQ ID NO:31, or a variant thereof having at least 95% sequence identity to the sequence set forth in SEQ ID NO:31.

Marzo *et al.* and Adrian *et al.* fail to disclose isolated human ANT1 polypeptides or variants thereof having at least 95% amino acid identity to the human ANT1 polypeptide sequence set forth in SEQ ID NO:31. Contrary to the PTO's assertion, Applicants submit that Marzo *et al.* fail to describe any isolated *human* ANT polypeptide, much less an isolated human ANT1 polypeptide, much less further an isolated human ANT1 polypeptide having the amino acid sequence of SEQ ID NO:31 or a variant having at least 95% sequence identity thereto. Marzo *et al.* merely describe ANT polypeptides isolated from *rat* myocardium (Fig. 4(c)).

Adrian *et al.* merely describe recombinant expression in yeast of homologous (*i.e.*, yeast-derived sequences), truncated yeast ANT fusion proteins. Adrian *et al.* fail, however, to provide any teaching of the expression of a recombinant human ANT1 polypeptide. The disclosure of Adrian *et al.* is directed to identifying N-terminal sequences of yeast ANT polypeptides that are responsible for mitochondrial localization, in yeast cells, of yeast ANT fusion proteins which merely comprise N-terminal fragments of yeast ANT, and which in any event are not human ANT polypeptides having at least 95% identity to SEQ ID NO:31.

Applicants additionally submit that the teachings of Wallace *et al.* fail to anticipate claim 43, and that Wallace *et al.*, Marzo *et al.*, and Adrian *et al.* all fail to anticipate the polypeptide fragment described in new claim 113 as introduced by the present amendment. Specifically, the disclosure of Wallace *et al.* fails to provide an isolated human ANT1 polypeptide fragment comprising at least 30 contiguous amino acid residues of the human ANT1 polypeptide sequence set forth in SEQ ID NO:31.

Accordingly, Applicants submit that the PTO has failed to establish anticipation of the instant claims by any of the cited documents and therefore respectfully request that in light of the above remarks and the present claim amendments, these rejections be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 103(a)

Claims 42-44, 47-50, 52-55 and 57 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Adrian *et al.* (1986 *Mol. Cell. Biol.* 6:626) in view of Fiore *et al.* (1998 *Biochimie* 80:137) and Marzo *et al.* (1998 *Science* 281:2027). The Action asserts that Adrian *et al.* disclose the expression of yeast ANT fusion proteins that are delivered to the mitochondria and are considered variants of ANT1, that Fiore *et al.* provide the amino acid sequence of human ANT1, and that Marzo *et al.* disclose the expression and isolation of highly purified recombinant ANT2. The Action alleges that it would have been obvious to one having ordinary skill in the art at the time of the claimed invention to express the ANT1 sequence disclosed by Fiore *et al.* as a fusion protein according to Adrian *et al.* The PTO further asserts that the ordinarily skilled artisan would have been motivated by the cited documents to substitute ANT1 instead of yeast ANT, and would also have had a reasonable expectation of success in the recombinant

production of an ANT1 fusion protein, based upon recombinant expression of human ANT2 according to Marzo *et al.*

Applicants respectfully traverse this rejection. As also noted above, cancellation of claim 44 by amendment herewith obviates the present rejection of this claim. Applicants submit that the instant claims are not obvious in light of the cited publications, which Applicants submit fail, alone or in combination, to disclose each element of the claimed invention. Therefore, the cited documents do not render obvious the instant claims, where it is well settled that in order to establish *prima facie* obviousness of a claimed invention, all of the claim elements must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981 (CCPA 1974).

In particular, Applicants submit that the cited publications, alone or in combination, fail to teach or suggest an isolated recombinant human ANT1 polypeptide or a variant or fragment thereof, as described in the specification and recited by the claims. Contrary to the assertion made by the PTO, none of the cited documents describes or suggests an isolated recombinant human ANT1 polypeptide having an amino acid sequence at least 95% identical to SEQ ID NO:31, nor a fragment thereof comprising at least 30 contiguous amino acids. Applicants submit that the instant claims are directed to isolated recombinant human ANT1 polypeptides and variants and fragments thereof, while the cited documents merely disclose recombinant yeast ANT fusion proteins (Adrian *et al.*), the amino acid *sequence* (but no isolated polypeptide *per se*) of ANT1 (Fiore *et al.*), and isolated non-recombinant rat ANT polypeptides (Marzo *et al.*). Applicants note that none of these publications describes the recombinant production or isolation of a human ANT1 polypeptide. In addition, Applicants note that the fusion proteins expressed from the two-hybrid constructs described by Marzo *et al.*, which comprise amino acid resides 105-156 of human ANT2, were <u>not</u> isolated but were merely implicated on the basis of β–gal activity in intact recombinant yeast (Marzo *et al.*, Fig. 4(b)), and that the isolated ANT proteins described by Marzo *et al.* were not human ANT2 polypeptides, as asserted by the Action. Rather, they were <u>non</u>-recombinant <u>rat</u> ANT proteins isolated from rat myocardium (*see* Marzo *et al.*, Fig 4(c)).

In addition, the PTO fails to establish that the skilled artisan, by combining the teachings of the cited publications, would have had a reasonable expectation of successfully expressing and isolating a recombinant human ANT1 polypeptide, or variant or fragment thereof.

Applicants submit that the skilled artisan would readily appreciate that the production of a functional human protein using a yeast-based expression system cannot be reasonably expected based upon the teachings of Adrian *et al.*, who merely describe the expression of yeast fusion proteins in yeast cells. As discussed further below and in the accompanying Declaration of Dr. Christen M. Anderson, the recombinant production of functional human ANT polypeptides has historically been very difficult, if not impossible. Accordingly, Applicants submit that the demonstration that a yeast ANT fusion protein could be expressed in yeast cells would not provide the skilled artisan with a reasonable expectation that a human ANT polypeptide could be successfully expressed in and isolated from yeast or other cells.

Furthermore, Applicants submit that the fusion proteins described in Adrian *et al.* comprise truncated yeast ANT polypeptide, which include less than 95% of the full length yeast ANT polypeptide. Applicants note that claims 42, 43, 47 and 51, and claims dependent therefrom, relate to isolated human ANT1 polypeptides that have at least 95% amino acid sequence identity to full length human ANT1. Indeed, as understood by the skilled artisan, the production of functional full length polypeptides is by no means always a routine procedure (*see, e.g.,* the enclosed Declaration of Dr. Christen M. Andersen). Accordingly, Applicants submit that the skilled artisan would not have had a reasonable expectation of successfully producing the claimed human ANT1 polypeptides and fusion proteins, having at least 95% identity to full length human ANT1, based upon the expression of truncated yeast ANT fusion proteins in yeast cells, particularly absent any evidence that these fusion proteins could be purified.

Applicants also respectfully point out that claim 42 is directed to an ANT1 polypeptide expressed from a construct comprising at least one regulated promoter operably linked to the nucleic acid encoding the polypeptide. As understood in the art and described in the instant specification, a regulated promoter is a promoter that may be treated in some manner so as to increase or decrease expression from an operably linked nucleic acid sequence. For instance, the instant specification teaches that a regulated promoter is an inducible promoter (*see, e.g.*, page 31, lines 4-18). Moreover, the specification specifically states, when describing a method of recombinant expression, that "the selected promoter, if it is a regulated promoter as provided herein, is induced by appropriate means (*e.g.*, temperature shift or chemical induction)" (page 29, lines 17-21).

Applicants submit that by contrast, Adrian *et al.*, Fiore *et al.* and Marzo *et al.* all fail to teach or suggest an ANT1 polypeptide recombinantly expressed from a regulatable promoter. To the contrary, Adrian *et al.* describe expression of yeast ANT-β-gal fusion proteins from a *constitutive* yeast promoter, and Marzo *et al.* describe expression (by implication only, using dihybrid β-gal reconstitution in intact yeast *without* isolation of any polypeptide, *see* comment re: Marzo *et al.* Fig. 4(b), *supra*) of a fusion protein comprising only amino acid residues 105-156 of human ANT2 from a *constitutive* yeast promoter. Fiore *et al.* fail to remedy this deficiency of Adrian *et al.* and Marzo *et al.*, since Fiore et al. fail to teach or suggest any recombinant expression of any human ANT polypeptide using any regulatable promoter. Accordingly, Applicants submit that since the combination of cited documents fails to teach or suggest each element of the claimed invention and, further, would not provide the skilled artisan with a reasonable expectation of successfully arriving at the present invention, the PTO has failed to establish a *prima facie* case of obviousness.

Even assuming *arguendo*, that each element of the presently claimed invention was described in the cited references, the mere fact that the teachings of the prior art *can* be combined or modified, or that a person having ordinary skill in the art is *capable* of combining or modifying the teachings of the prior art, does not make the resultant combination *prima facie* obvious, as the prior art must also suggest the desirability of the combination (*see, e.g., In re Mills*, 16 USPQ2d 1430, Fed. Cir. 1990; *In re Fritch*, 23 USPQ2d 1780, Fed. Cir. 1992). Applicants submit that the prior art absolutely does not teach or suggest the desirability of combining the references to achieve the presently claimed invention drawn to recombinant human and animal ANT1 polypeptides, and, therefore, the Action fails to establish a *prima facie* case of obviousness.

Claims 51 and 56 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Adrian *et al.*, Fiore *et al.*, and Marzo *et al.*, as applied to claims 42-44, 47-50, 52-55 and 57 above, and further in view of Rosenberg (1996 *Protein Analysis and Purification: Benchtop Techniques*, Birkhauser, Boston, pp. 335-347). More specifically, the Action alleges that Rosenberg shows that it is standard in the art to construct fusion proteins comprising a protein of interest and an enzyme or affinity tag. In addition, the Action asserts that Rosenberg teaches that a protease cleavage site can easily be engineered into the fusion protein so that the fusion partner

can be separated from the protein of interest after purification. The Action asserts that it would have been obvious to one of ordinary skill in the art at the time of the invention to make a cleavable fusion protein between ANT1 and an enzyme, based upon Rosenberg's disclosure and in light of teachings by Adrian *et al.* that the fusion partner may be interfering with the mitochondrial localization sequences within yeast ANT-β-gal fusion proteins.

Applicants respectfully traverse this rejection and submit that the combination of cited publications fails to teach or suggest each element of the claimed invention. For reasons given above, Applicants submit that the cited documents, alone or in combination, fail to teach or suggest an isolated recombinant ANT1 fusion protein. As also discussed above, the ordinarily skilled artisan could not have had a reasonable expectation of successfully producing the claimed recombinant ANT1 fusion proteins, given the teachings of the cited documents. Additionally and importantly, Applicants submit that there was clear recognition by the art that recombinant human ANT polypeptides were notoriously difficult to produce, as described below and in the accompanying Declaration of Christen M. Anderson. Applicants further submit that the addition by the PTO of Rosenberg as a cited publication fails to remedy the deficiencies of Adrian *et al.*, Fiore *et al.* and Marzo *et al.*, even in combination with any other prior publications, since Rosenberg also fails to provide any reasonable expectation of successfully achieving recombinant expression of a human ANT polypeptide or fusion protein.

The documents cited by the PTO merely describe fusion proteins comprising yeast ANT polypeptides, or a construct encoding a fusion protein (isolation of which is nowhere contemplated, *see* comment re: Marzo *et al.*, Fig. 4(b), *supra*) comprising amino acid residues 105-156 of human ANT2. Applicants submit that the skilled artisan would appreciate that the difficulties associated with the recombinant expression of a polypeptide typically increase as the length of the polypeptide increases, and/or as the structural complexity of the polypeptide increases. Human ANT1, as described in the present application (*e.g.*, page 18, lines 1-27 including cited SEQ ID NOS, Figures and references), comprises an amino acid sequence of 297 residues and includes multiple hydrophobic regions. Thus, at the priority filing date of the present application a person having ordinary skill in the art would not reasonably have expected successfully to express recombinant human ANT1 simply by engineering a full length (or at least

95% of full length) ANT1 encoding polynucleotide into any routine recombinant expression construct using any routine recombinant expression methodology.

Furthermore, Applicants submit that the skilled artisan recognizes that it is generally more difficult to express and isolate functional polypeptides derived from higher eukaryotes (*e.g.*, humans) than from lower organisms, (*e.g.*, bacteria, yeast), due to complexities associated with the former, such as preferential codon utilization, differences in tRNA pools, co- or post-translational modifications, *etc.* Accordingly, Applicants submit that the skilled artisan would not have been motivated, with the requisite reasonable expectation of success, to produce isolated human or animal ANT1 fusion proteins comprising full length ANT1 polypeptides. In particular, no such motivation would have arisen based on descriptions in the cited publications of the mere expression in a homologous (*i.e.*, yeast ANT in yeast expression host) system of fusion proteins comprising yeast ANT polypeptides, or of the expression (but not the isolation, presumably due to insufficient amounts--detection was only through signal amplification afforded by dihybrid reconstitution of β-gal; *see* comment, *supra*, re: Marzo *et al.*, Fig. 4(b), *see also* Anderson Declaration) of a grossly truncated ANT fragment, *i.e.*, only 52 amino acid residues of the human ANT2 polypeptide. Accordingly, Applicants respectfully request that this rejection be withdrawn.

In addition to the above remarks, Applicants also respectfully submit that the present invention is nonobvious when "secondary factors," including, in particular, the identification of a long-felt need and the failure of others, are considered. It is well established that considerations such as long-felt but unsolved needs, and the failure of others to arrive at applicants' invention, are not only relevant to the obviousness inquiry, but must be considered when present. *Custom Accessories Inc., v. Jeffrey-Allan Industries Inc.*, 807 F.2d 955; 1 USPQ2d 1196 (Fed. Cir. 1986); *Ryko Manufacturing Co. v. Nu-Star Inc.*, 950 F.2d 714, 21 USPQ2d 1053, 1057 (Fed. Cir. 1991).

As evidence of the existence of secondary factors demonstrating that the present invention was not obvious at the time of filing the instant application, Applicants submit herewith the Declaration of Dr. Christen M. Anderson, which presents evidence of the importance of ANT polypeptides in human disease, the long-felt need for recombinant ANT polypeptides for additional research, and the unsuccessful attempts by other investigators to

produce recombinant ANT polypeptides. In light of the Declaration, Applicants respectfully submit that cDNA sequences encoding a human ANT polypeptide were known as early as 1987, and recombinant protein expression methods were established well before 1987. In addition, the desirability of expressing a functional ANT polypeptide is clearly evidenced by the attention directed to ANT polypeptides by numerous investigators, as indicated by the references cited throughout the instant specification. Accordingly, Applicants submit that a long-felt need for the reliable expression of ANT polypeptides was present at the time of filing the parent of the instant application in 1998. Moreover, Applicants are unaware of any successful production by others of a functional isolated recombinant human ANT polypeptide, according to the instant invention. In view of the absence of any such disclosures in the prior art, and further in view of the unsuccessful efforts to express recombinant ANT polypeptides, as described in the Declaration, Applicants respectfully submit that the present invention is nonobvious in light of the long-felt unmet need for recombinant ANT polypeptides and fusion proteins.

In view of the foregoing, Applicants respectfully submit that the Action has not established a *prima facie* case of obviousness. Applicants submit that the cited documents fail to teach or suggest each element of the claimed invention, and also fail to provide a suggestion or motivation to a person having ordinary skill in the art to modify or combine the prior art teachings to arrive at the claimed invention with a reasonable expectation of success. Furthermore, as discussed above, secondary considerations clearly indicate the invention to be non-obvious. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Additionally, applicants wish to call the Examiner's attention to several related co-pending applications having claims potentially directed to similar subject matter. Reference to the appended "Table of Co-Pending Applications" is therefore requested.

The Commissioner is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Applicants respectfully submit that all of the claims remaining in the application are now allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Christen M. Anderson et al.

SEED Intellectual Property Law Group PLLC

Stephen J. Rosenman, Ph.D.
Registration No. 43,058

SJR:kw

Enclosure:
Postcard
Check
Copy of Miroux et al.
Petition for Extension of Time

701 Fifth Avenue, Suite 6300
Seattle, Washington 98104-7092
Phone: (206) 622-4900
Fax: (206) 682-6031

435655_1.DOC

APPENDIX: TABLE OF CO-PENDING APPLICATIONS

U.S.A.N.	Atty. Docket No.	Examiner	Claims directed to ____ (Comments)
09/393,441	660088.420C1	Sheridan Snedden	__ isolated recombinant huANT3 polypeptide that localizes to mitochondrial membrane Statutory double-patenting rejection of claims 42, 46-48, 51 and 57 over claims 42, 46-48,51 and 57 of 09/185,904
09/185,904	660088.420	Holly G. Schnizer	__ isolated recombinant huANT3 polypeptide Obviousness-type double patenting rejection of claims 42, 46-50 over claims 42, 46-48, 51 and 57 of 09/393,441
09/811,131	660088.420D1	Holly G. Schnizer	__ method of identifying agent that binds to ANT polypeptide
09/811,185	660088.420D2	Rebecca L. Anderson	__ method of treatment using ANT ligand
09/810,644	660088.420D3	Rebecca L. Anderson	__ ANT ligand
09/811,094	660088.420D4	Holly G. Schnizer	__ recombinant expression construct, host cell, and method of making recombinant ANT polypeptides and fusion proteins
09/811,132	660088.420D5	Holly G. Schnizer	__ methods of detecting and isolating an ANT polypeptide, using ANT ligand
09/809,827 (present application)	660088.420D6	Holly G. Schnizer	__ isolated recombinant huANT1 polypeptide
09/809,889	660088.420D7	Holly G. Schnizer	__ isolated recombinant huANT2 polypeptide
09/569,327	660088.443	Sheridan Snedden	__ method of producing recombinant ANT polypeptides and fusion proteins using tightly regulated promoter
10/684,232	660088.433C2	(none assigned)	__ANT-energy transfer peptide fusion proteins